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LIPID PEROXIDATION—A FACTOR IN ANOXIA INTOLERANCE IN *IRIS* SPECIES?

M. IAN S. HUNTER*, ALISTAIR M. HETHERINGTON and ROBERT M. M. CRAWFORD

Department of Botany, *Department of Biochemistry and Microbiology, University of St Andrews, Fife, U K.

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Key Word Index—*Iris pseudacorus, I germanica*; Iridaceae; rhizome, anoxia, lipid peroxidation; malondialdehyde

Abstract—Fourteen days anoxia resulted in a 38-fold increase in the level of the lipid peroxidation product, malondialdehyde in the anoxia-intolerant *Iris germanica*, compared with aerobic controls. Six hours exposure to air after anoxic treatment resulted in an even greater (157-fold) increase in malondialdehyde. By contrast, in the closely related, anoxia-tolerant species, *I. pseudacorus*, malondialdehyde levels declined slightly after anoxic treatment. Increased lipid peroxidation following re-exposure to air may be a significant factor in the lethality of anoxia to *I. germanica*.

INTRODUCTION

The ability of certain higher plants to withstand prolonged periods of anoxia is particularly important (and undoubtedly confers a selective advantage) in habitats prone to flooding. The genus *Iris* contains morphologically similar species which differ markedly in their ecological preference for wet or dry sites. *Iris germanica* L. (var Quechei) is a cultivated variety, probably of Mediterranean origin, and is typically found in well-drained soils. *Iris pseudacorus* L., however, occupies habitats such as lakeside muds characterized by poor oxygen availability. Even during midsummer, oxygen concentrations within the rhizome are low (2 7% v/v) [1] but during the winter, following shoot dieback, and interruption of oxygen transport to the rhizome, there must be extensive periods of anoxic stress within that organ. We have demonstrated [2] that *I pseudacorus* is capable of surviving complete anoxia for at least 8 weeks, whereas *I. germanica* is much less able to tolerate these conditions.

The lethal target for anoxic damage is as yet unclear but since molecular oxygen is required as the terminal electron acceptor for aerobic respiration and also for processes such as fatty acid desaturation [3] and sterol biosynthesis [4] altered energy metabolism or membrane lipid biosynthesis might well be important factors in the pathology of anoxia. Examination of the effect of anoxia on lipid composition in these two *Iris* species [5] revealed no significant changes in *I. germanica* but losses of polar lipids, especially saturated fatty acids, in the tolerant *I. pseudacorus* These results were surprising, as anoxia might be expected to lead to losses of unsaturated acids, but nevertheless have important consequences for membrane function and may represent some form of adaptation to anoxia [5].

One hitherto little investigated mechanism for cellular damage after periods of anoxia may be membrane lipid peroxidation. Peroxidative damage underlies a number of membrane pathologies [6] and in plants has been implicated in such processes as leaf senescence [7], wounding [8] and susceptibility to drought [9]. Protective mechanisms against such damage, such as catalase, peroxidases and superoxide dismutase, exist and the latter is inducible by oxygen in plants, animals and bacteria [10] and, therefore, undoubtedly declines during anoxia, so that the critical phase of damage may not be during the anoxic period itself but immediately after re-exposure to oxygen when defences may be inadequate

The purpose of this investigation was to assess the degree of lipid peroxidation occurring in the two *Iris* species after anoxia by measuring the amounts of the lipid peroxide breakdown product, malondialdehyde (MDA). The primary shoot was chosen as the region for investigation since it appeared particularly sensitive to anoxia in the intolerant species, *I. germanica* [2].

RESULTS AND DISCUSSION

Tables 1 and 2 show the amounts of MDA measurable after the different treatments, expressed on the basis of dry wt, fr. wt and total lipid. It is significant that the general trends in MDA are the same irrespective of the method of calculation since this eliminates the possibility that differences in moisture or total lipid content account for the alterations in MDA which occur

Irrespective of treatment, the MDA concentration is much lower (especially in the aerobic control) in *I. germanica* than in *I. pseudacorus*, suggesting an increased level of lipid peroxidation in the latter. An important factor in the susceptibility of membrane systems to peroxidation has been shown to be the proportion of polyunsaturated fatty acids esterified to polar lipids [11] In whole rhizomes, however, there is little difference in the relative proportions of the polar lipid fatty acids of the two *Iris* species under aerobic conditions [5]. Furthermore, the total lipid and polar lipid content (on a dry wt basis) of *I. germanica* whole rhizomes is considerably greater than that of *I pseudacorus*. However, in the present work attention has been focused on the terminal shoot which may well have a different lipid composition since the different cell types in different anatomical regions are likely to contain different propor-

Table 1 Malondialdehyde concentrations related to fr wt, dry wt and total lipid in primary shoots of *Iris pseudacorus* rhizomes subject to varying degrees of anoxia

Treatment	nmol MDA/g fr wt	Ratio*	nmol MDA/g dry wt	Ratio*	nmol MDA/g lipid	Ratio*
Anoxia	0 638	0 56	4 95	0 88	65 0†	0 96
	(0 080)		(0 75)		(10 0)	
Anoxia +	0 795	0 69	4 90	0 88	82 5	1 22
6 hr air	(0 095)		(0 25)		(17 5)	
Aerobic	1 15		5 58		67 5†	

*Ratio = anoxic treatment/aerobic control Each value is the mean of five replicates except those marked † where $n = 4$

Figures in parentheses are s e

Table 2 Malondialdehyde concentrations related to fr wt, dry wt and total lipid in primary shoots of *Iris germanica* rhizomes subjected to varying degrees of anoxia

Treatment	nmol MDA g ft wt	Ratio*	nmol MDA/g dry wt	Ratio*	nmol MDA g lipid	Ratio*
Anoxia	0 0770	32 49	0 583	37 61	6 18	27 96
	(0 0017)		(0 015)		(1 38)	
Anoxia +	0 330	139 24	2 43	156 77	35 0	158 37
6 hr air	(0 065)		(0 60)		(7 5)	
Aerobic	0 00237		0 0155		0 221	
	(0 00066)		(0 0044)		(0 045)	

*Ratio = anoxic treatment/aerobic control Each value is the mean of five replicates Figure in parentheses are s e

tions of subcellular organelles each with their own characteristic membrane lipid composition [12]

As can be seen from Tables 1 and 2, after 14 days anoxia, there is a slight decrease in MDA in *I pseudacorus* with little difference after exposure of the anoxia-treated rhizomes to an air atmosphere for 6 hr In complete contrast there is a 38-fold increase (on a dry wt basis) in MDA in *I germanica* rhizomes kept under anoxia even before re-exposure to air, but after 6 hr in air the concentrations rise to some 157-times those of the aerobic control A similar increase in lipid peroxidation in apical buds of *Phaseolus vulgaris* stored under nitrogen (22 hr) and then incubated in air (0 5 hr) has also been reported [13] A fascinating parallel is to be found in mammalian tissues where lipid peroxidative membrane damage is demonstrable as a major factor in the pathological changes accompanying post-hypoxic re-oxygenation after ischaemia [14, 15]

Although the absolute amounts of lipid peroxidation appear higher in *I pseudacorus* than in *I germanica*, even after re-exposure to air after anoxia, the fact that there is little change in the former may well indicate that the activity of one or more protective enzymes is not altered significantly by anoxia On the other hand, the striking increase in lipid peroxidation seen in *I germanica*, especially after re-exposure to air, may be a reflection of the decline of such a protective mechanism during the anoxic period Likely candidates for these enzymes are superoxide dismutase and catalase since these destroy O_2^- and hydrogen peroxide, respectively, which are known to be involved in the evolution of the highly reactive and toxic species, hydroxyl radicals and singlet oxygen, both of which are potent initiators of lipid peroxidation [16] In support of this suggestion both enzymes show an inverse correlation with lipid peroxidation and consequent membrane damage in leaf senescence [9] and drought intolerance [7] Superoxide dismutase and glutathione peroxidase (another important hydrogen peroxide destroying enzyme) also decline in hypoxic mammalian tissue and in which markedly increased lipid peroxidation accompanies re-oxygenation [14] As oxygen is essential for the later stages of lipid peroxidation, following the formation of free-radical intermediates by initiators of the process but preceding the evolution of MDA from lipid hydroperoxides, [16] it is perhaps surprising that the concentrations of MDA increase in *I germanica* rhizomes subjected to anoxia but not re-exposed to air However, if there is a decline in one or more protective mechanisms during anoxia then it is possible that sufficient oxygen remains in the tissue to bring about significant lipid peroxidation. Alternatively, it is possible that free radical intermediates formed in the absence of oxygen may react during the TBA test to yield TBA-reactive material [17]

If, as we suggest, lipid peroxidation is a significant factor in the failure of *I germanica* to survive anoxia, then this species must be more sensitive to its damaging effects than *I pseudacorus* which can clearly tolerate higher levels without any decline in primary shoot viability [2] Such tolerance could be conferred by an active system for removal and replacement of peroxidized side chains of polar lipids and the fact that membrane lipid compositional changes do accompany anoxia in this species [5] may be indicative of such a repair mechanism

EXPERIMENTAL

The sources of the *Iris* species, growth conditions, preparation for aerobic and anaerobic treatments were all as described

previously [2] After 14 days, all rhizomes were harvested and treated in the following manner: a segment (*ca* 0.75 cm^2) of tissue was rapidly excised from the meristematic region of the cut primary shoot *Ca* one-third of this was used for the thiobarbituric acid (TBA) reaction whilst the remainder was immediately ground in liquid N_2 and freeze-dried overnight Aerobic control ($n = 5$) and anaerobic rhizomes ($n = 5$) were treated as above immediately after harvesting A further five anaerobic rhizomes were exposed to an air atmosphere for 6 hr before processing The anaerobic rhizomes were harvested and processed within the anaerobic work-bench (Forma Scientific, Ohio, U S A), except for liquid N_2 treatment

TBA reaction Carried out by the methods of refs [9, 18] but with the introduction of a BuOH extraction step (removal of interfering compounds [19]), and the inclusion of Fe^{3+}, which is important to colour development, and butylated hydroxytoluene (BHT) to prevent further peroxidation [20] The final method used was as follows tissue (*ca* 550 mg fr wt) was ground in 5 ml 72% TCA To this was added 5 ml TBA (0 5%), 0 23 ml $FeCl_3 \cdot 6H_2O$ (0.29%), 0 23 ml BHT (0 23% in EtOH) and the mixture heated for 1 hr at 95° After rapid cooling at 0°, 4 ml *n*-BuOH was added and, following vortex mixing, the extract was centrifuged to ensure complete separation into two phases. The *A* of the BuOH supernatant was determined at 535 and 520 nm and the MDA concn calcd from the difference in the two *A*s [19] and a molar coefficient of extinction for MDA of $1\,56 \times 10^5$/M cm [21]. For the anaerobic rhizomes, all steps prior to heating were carried out in the anaerobic workbench

Lipid extraction. Total lipid was extracted from the freeze-dried material, after rehydration with H_2O (3 ml/g) using hexane–*iso*-PrOH [22]

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